U.S. Fish & Wildlife Service

Mussel Survey Recommendations

Dwarf Wedgemussel

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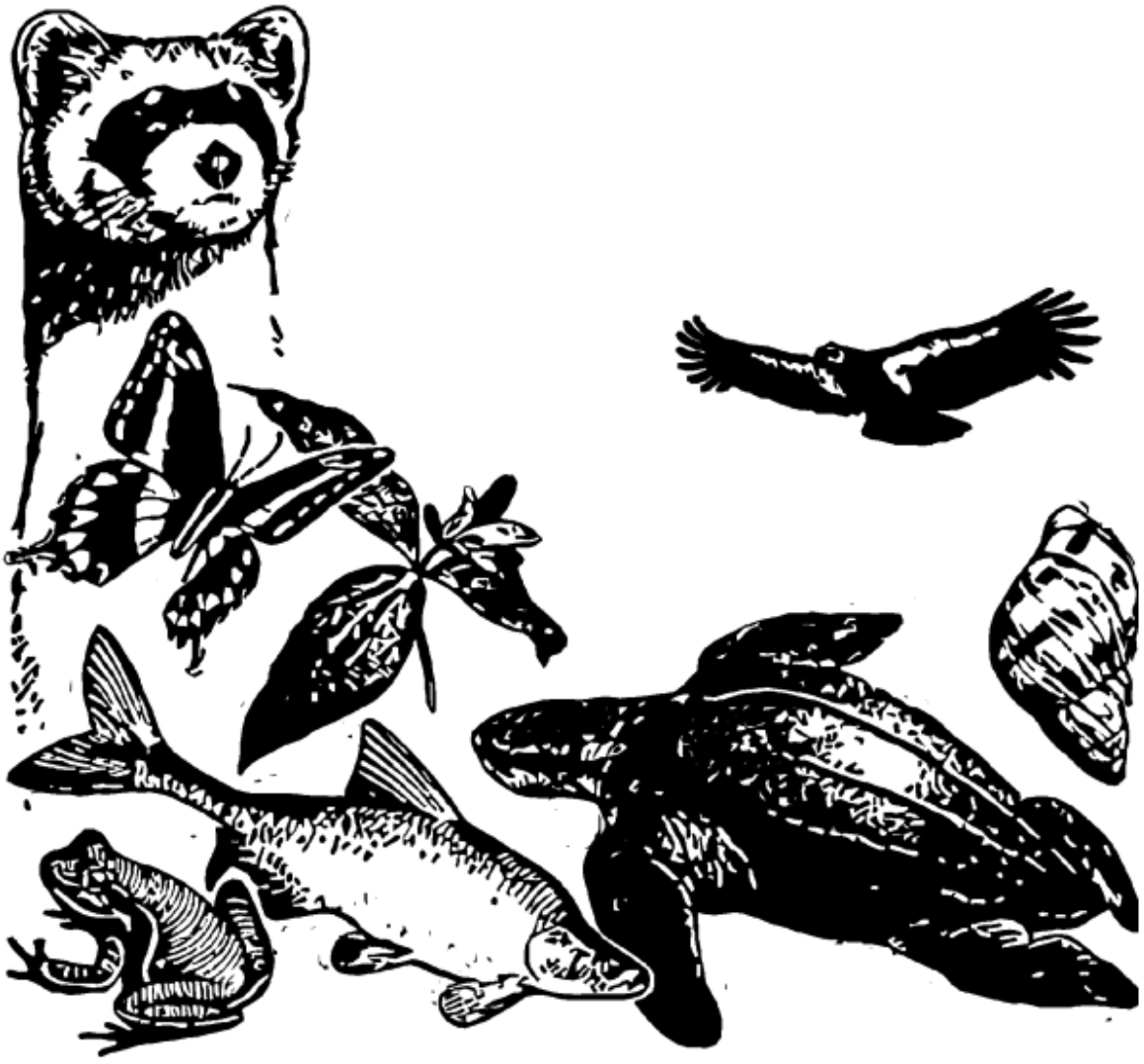

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David R. Smith; Rita F. Villella; David P. Lemarie

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Survey protocol for assessment of endangered freshwater mussels in the Allegheny River, Pennsylvania

DAVID R. SMITH,[1] RITA F. VILLELLA, AND DAVID P. LEMARIÉ

US Geological Survey, Biological Resources Division, Leetown Science Center, 1700 Leetown Road, Kearneysville, West Virginia 25430 USA

Abstract. The United States Endangered Species Act (ESA) requires a biological assessment of any activity that is authorized, funded, or carried out by a federal agency and likely to affect a federally listed endangered species or its critical habitat. We developed a standardized survey protocol for biological assessments of the effects of bridge replacements on 2 federally listed endangered freshwater mussels, *Epioblasma torulosa rangiana* and *Pleurobema clava*, found in the Allegheny River, Pennsylvania. The protocol combines qualitative sampling to determine species present with quantitative sampling to estimate density. Data on species present satisfy the minimum requirement of a biological assessment, whereas estimates of density are needed to assess the number of individuals that would die as a result of bridge replacement. Some excavation of substrate is necessary for unbiased population estimates because of species and sex-specific differences in detection at the substrate surface. We reduced the amount of excavation and cost of the survey by using a statistical sampling technique called double sampling, which uses counts from excavating a subset of quadrats to calibrate counts from searching the substrate surface of all quadrats. We applied the survey protocol to the Allegheny River at West Hickory where *E. t. rangiana* was the 3rd and *P. clava* was the 4th most abundant mussel at the site. Only 31% of *P. clava* and 52% of *E. t. rangiana* (80% of females, 45% of males) were detected at the substrate surface. We estimated that 9173 (95% CI: 6309–13,336) *E. t. rangiana* and 7010 (95% CI: 4462–11,013) *P. clava* lived within 50 m of the existing bridge and would be affected immediately by bridge construction. (Population estimates did not include mussels too small to be retained on a 6.35-mm-mesh sieve.) Application of the protocol is not limited to biological assessment under the ESA, but is appropriate where site-specific status of freshwater mussel populations is required.

Key words: freshwater mussels, population assessment, sampling design, *Epioblasma torulosa rangiana*, *Pleurobema clava*, Endangered Species Act.

The Allegheny River drainage supports some of the largest remaining populations of *Pleurobema clava* and *Epioblasma torulosa rangiana*, 2 freshwater mussel species listed as endangered by the US Fish and Wildlife Service (1994). The Pennsylvania Department of Transportation (PennDOT) plans to replace a series of older bridges along the Allegheny River within the species' current range. Bridge replacement will be undertaken with funds from the Federal Highway Administration, so biological assessments are required as stipulated under the Federal Endangered Species Act (ESA).

Biological assessment evaluates the potential effects of a federal activity on federally listed species to determine whether formal consultation is necessary (US Fish and Wildlife Service and National Marine Fisheries Service 1998). Formal consultation determines whether a proposed activity is likely to jeopardize the continued existence of a federally listed species or adversely affect designated critical habitat (US Fish and Wildlife Service and National Marine Fisheries Service 1998). A biological assessment includes an on-site inspection to determine species present in the area to be affected by the federal activity and an analysis of the potential effects on the species and its habitat. A biological assessment is to be based on information that is reliable, credible, and represents the best scientific and commercial data available (US Fish and Wildlife Service and National Marine Fisheries Service 1998). However, the contents and methods of the biological assessment are left to the discretion of the Federal agency that is funding the activity.

We present a standardized survey protocol for the biological assessment of *E. t. rangiana* and *P. clava* at bridge replacement sites on the Allegheny River. The protocol combines qualitative sampling to determine species present with quantitative sampling to estimate density. Data on species present satisfy the minimum requirement of a biological assessment, whereas

[1] E-mail address: david_r_smith@usgs.gov

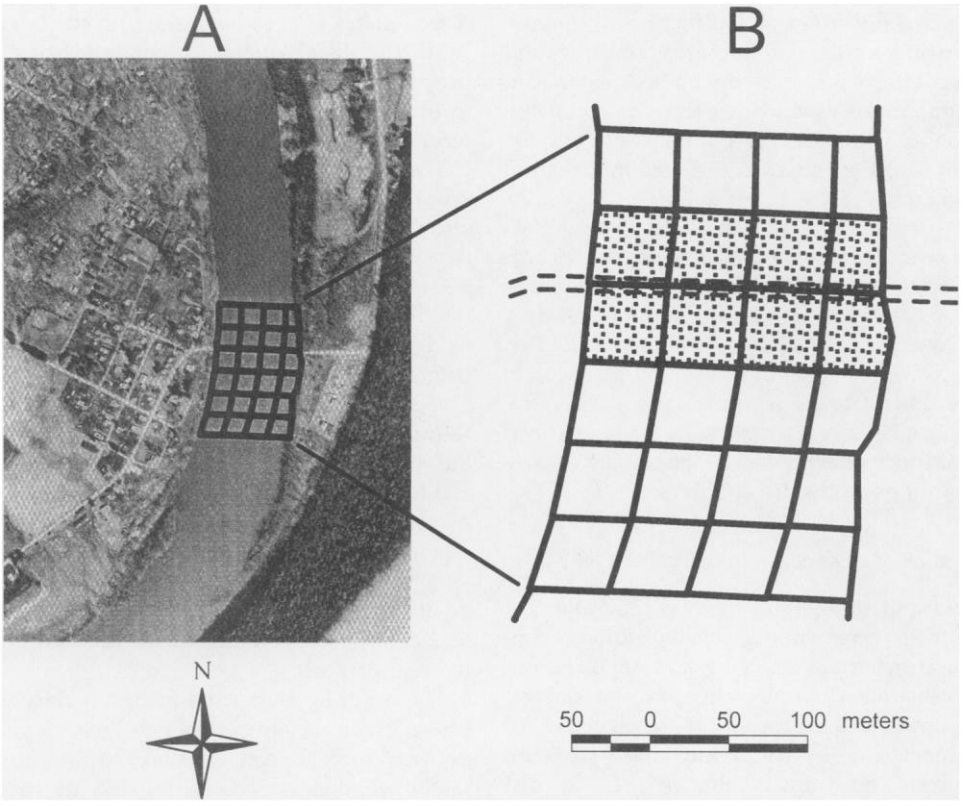

FIG. 1. Location of the study site. A.—Portion of a digital orthophoto quarter quadrangle showing West Hickory on the west bank of the Allegheny River. B.—Grid of 24 cells in 6 rows and 4 columns overlaid on a stretch of the Allegheny River and used for timed searches of freshwater mussels. The dashed lines show the position of the bridge. The small squares in the 4th and 5th rows from the bottom show location of quadrats in the direct-effects area; the remainder of the site is the indirect-effects area. Water flow is to the south.

estimates of abundance are needed to assess the number of individuals that may be negatively affected by bridge replacement. We applied the protocol to the Allegheny River at West Hickory, Pennsylvania, where bridge replacement was proposed. The protocol may be useful at other sites where an assessment of rare freshwater mussels is needed.

Methods

The bridge links Harmony Township to Hickory Township in Forest County (lat 41.574°N, long 79.411°W; Fig. 1). A previous survey (Aquatic Systems 1998) detected *P. clava* and *E. t. rangiana*, but did not estimate density or abundance. We designed a survey protocol as if the West Hickory site had not been surveyed previously because our protocol would be used at other bridge sites along the Allegheny River where no surveys had been done.

Survey protocol

We wanted to determine species present, estimate population density, and estimate size structure to indicate recent recruitment. Effects of construction on mussels, which include mortality, displacement, and interference with growth or reproduction, can stem *directly* from the construction action or occur *indirectly*. An example of a direct effect would be burial under a causeway, and an example of an indirect effect would be change in habitat upstream of a causeway as a result of pooling. High mortality, displacement, or interference with growth or reproduction are certain as a result of direct effects, but their likelihood as a result of indirect

effects are uncertain and difficult to quantify. We partitioned the site into areas of direct and indirect effects and determined species present throughout the site. However, we estimated abundance and size distribution only for the area of direct effects to assess certain loss from construction.

We surveyed the site on 12 to 15 July 1999 when river flow was low and water clarity was high. High proportions of some mussels are at the substrate surface during summer (Amyot and Downing 1991, Balfour and Smock 1995).

There were 3 steps in the survey protocol: 1) delineation of areas of direct and indirect effects, 2) qualitative sampling in areas of direct and indirect effects, and 3) quantitative sampling in the area of direct effects.

Delineation of areas of direct and indirect effects

Road and bridge construction may alter the physical environment (e.g., by deposition of construction materials, scouring and deposition of river substrate, changes in flow, erosion of river bank, and increased turbidity), the chemical environment (e.g., by runoff carrying petroleum products), and animal behavior (e.g., by displacement of fish that host glochidia after habitat loss) (Trombulak and Frissell 1999). Severity and spatial extent of the effects will depend on construction practices, timing of construction activities, river flows, composition of substrate, and effectiveness of erosion controls.

The area of direct effects was where mussels were likely to die or be displaced during or shortly after construction activities. Preliminary engineering plans involve constructing a causeway, and dropping the existing bridge into the river and partly on the causeway before removal. Bottom width of the causeway will depend on its surface elevation and top width. However, the proposed bottom width of a causeway at a similar bridge replacement project (Kennerdell, Pennsylvania, ~82 km downstream from West Hickory) was as wide as 41 m. The project at Kennerdell is also on the Allegheny River and similar to the West Hickory bridge replacement in size and proposed construction methods. The exact location and area of disturbance of the causeway at West Hickory will depend on the final bridge design, but the causeway will likely be offset from the centerline of the existing bridge. Thus, we added a buffer and judged the direct-effects area to be in the immediate vicinity of the disturbance (i.e., below existing and proposed bridges and causeways) extending 50 m upstream and 50 m downstream of the centerline of the existing bridge (Fig. 1).

The indirect-effects area was limited to likely scouring, sedimentation, and pooling from construction-related changes in river flow, and was 50 to 100 m upstream of the bridge and 50 to 200 m downstream of the bridge (Fig. 1), based on the hydrologic and hydraulic analysis for bridge replacement at Kennerdell (Parsons, Brinckerhoff, Quade, and Douglas, Inc. 1997). The report predicted that flow velocity would return to preconstruction levels across most of the river channel within 200 m downstream and 100 m upstream of the bridge. Thus, we determined that adverse effects at West Hickory would be contained within a similar 300 m stretch. The Allegheny River at West Hickory is, on average, 187.5 m wide so the study area was 56,250 m^2 (direct effects: 18,600 m^2, indirect effects: 37,650 m^2).

The exact impacts from bridge replacement, especially in the indirect-effects area, cannot be known prior to construction, so we do not know whether our reasoning led us to adequately encompass the spatial extent of the impacts. Only through follow-up monitoring at multiple sites can the spatial and temporal scales of the impacts be measured and the protocol be refined.

Qualitative sampling in areas of direct and indirect effects

Qualitative sampling included a search for piles of shell material (i.e., shell middens) discarded by mussel predators and a timed search for live mussels. Both banks were searched for middens along the entire study area and near the bases of the bridge piers, and locations of middens were mapped. To determine relative abundance and species composition in the middens, we identified species and counted valve pairs.

We divided the survey area into smaller areas or *cells* to do the timed search (Fig. 1). We defined *effective sampling fraction* as the % of a cell that is searched thoroughly, and based cell dimension on this fraction. Effective sampling fraction can be calculated by:

$$\frac{\text{effective search rate}\left(\frac{m^2}{\text{min}}\right) \times \text{search time (min)}}{\text{cell area } (m^2)}$$

where *effective search rate* is the area that can be searched thoroughly per min, and *search time* is the sum of time searched by all observers in a cell. We assumed an effective search rate of 0.5 m^2/min and selected a cell area of 2500 m^2 (cell dimensions = 50 m X 50 m) and a search time of 240 min/cell (e.g., 6 observers searching for 40 min each or 4 observers searching for 60 min each). This combination resulted in an effective sampling fraction of ~0.05. Effective sampling fraction can be used as a basis for standardizing and comparing qualitative searches. We established a marked grid in the river of 24 cells (4 columns and 6 rows) where 18 cells were 50 m wide and 6 cells (the 4th column) were of varying widths (Fig. 1). We typically deployed 3 teams of 4 observers allowing 3 cells to be surveyed simultaneously. Thus, each of the 4 observers spent a minimum of 60 min covering 1/4 of the cell. Search times were prorated for cells that were <50 X 50 m.

We snorkeled in wadeable water (<1.0–.5 m deep) and used SCUBA in depths >1.0 to 1.5 m, depending on turbidity and river flow. We snorkeled beginning at the downstream end of the cell to avoid disturbing sediment and reducing visibility. With SCUBA, we began at the upstream end of the cell to minimize exertion and air usage. We assumed equal search efficiency for divers and snorkelers. However, if there was a known difference in effective search rate then search times could be adjusted so that effective sampling fractions would remain equal for dived and snorkeled cells. Observers recorded location, cell dimensions, species counts, and actual search time for each cell. Each observer fanned away fine sediment, removed loose, nonembedded material, and raked loose sediment with fingertips in an effort to detect mussels.

Quantitative sampling of the area of direct effects

We quantitatively sampled to estimate abundance and to assess uncertainty in that estimate. There are many statistically valid sampling designs from which to choose (Thompson 1992, Dorazio 1999), but we used a double sampling design with 0.25-m^2 quadrats, systematically placed with multiple random starts, and excavation of a random subset of the quadrats (Smith et al. 2001). Systematic sampling is efficient for clustered and rare populations, provides good spatial coverage, and is easy to implement in field sampling (Murthy and Rao 1988, Thompson 1992, Christman 2000). Multiple random starts are important for variance estimation (Hedayat and Sinha 1991). Double sampling increases precision of density estimates for reduced cost by using total counts from a random subset of quadrats to calibrate surface counts from all quadrats.

Not all mussels can be observed on the substrate surface (Miller and Payne 1988, Amyot and Downing 1991, Balfour and Smock 1995, Smith et al. 2001), so we included excavation in the sampling protocol. Some excavation is required to eliminate observation bias, but it is usually inefficient to excavate all quadrats in a sample unless a low % (<40%) of mussels are detectable at the substrate surface (Smith et al. 2001). Use of a double sampling design reduces the amount of excavation, and therefore cost, required to achieve precise estimates (Smith et al. 2001). The 1st phase in the double sampling design includes a large sample (at least 100 in mid-Atlantic and northeastern US rivers: see below) of 0.25-m^2 quadrats within which only mussels on the substrate surface are counted. A representative subsample is selected from the 1st sample of quadrats for excavation; the size of the subsample depends on the expected proportion of mussels on the substrate surface. The excavated quadrats provide paired surface and total (= count below the surface + count at the surface) counts that are used to calibrate the surface counts for the entire sample. Calibration of the surface counts is done through a regression estimator, which is appropriate provided that the relation between surface and total counts is approximately linear (Hedayat and Sinha 1991). The linearity assumption can be examined with scatterplots. Formulae for estimating density and abundance for the recommended sampling design are presented in the Appendix.

For a double sampling survey with fixed total cost, Smith et al. (2001) found that the proportion of excavated quadrats that minimized variance of the density estimate depended on the expected % of mussels at the substrate surface. This relationship led to the following guidelines for determining the proportion of quadrats to excavate in the double sampling design (Smith

et al. 2001): if >60% of the mussels are likely to be detected at the surface then excavation of 25% of the quadrats will minimize variance; 50 to 60% = 33% of the quadrats, 40 to 50% = 50% of quadrats, and <40% = 100% of quadrats. We observed 50% of *P. clava* and 66% of *E. t. rangiana* at the substrate surface in August 1997 at Kennerdell, so we excavated 33% or every 3rd quadrat.

We placed quadrats in the site systematically after 3 random starts (Fig. 1) resulting in 3 systematic samples. Each systematic sample began at a randomly chosen location in the corner of the site (i.e., a random start) followed by a series of locations at equally spaced intervals. One concern with systematic sampling involves the possibility of finding the same number of mussels in all systematic samples. This event causes a variance estimate equal to 0, in which case we recommend an approximate variance formula (Appendix). Three random starts are small enough that implementing the systematic sample is still relatively easy, but large enough that finding equal numbers in all systematic samples is rare.

We selected intervals between systematically placed quadrats in the *across river* (d_1) and *up river* (d_2) directions. For each random start, we generated a pair of random numbers: from 0 to d_1 and from 0 to d_2, which defined the starting location of the 3 systematic samples. We then placed quadrats at the preset intervals. This design is called an aligned systematic sample with multiple random starts (Bellhouse 1988).

Intervals between systematically placed quadrats depend on the size of the direct-effects area, sample size, quadrat size, and number of random starts. To find equal intervals, we used the following algorithm: let n' be sample size and $n'_i = n'/k$, where $i = 1, \ldots, k$ is the number of quadrats in each of the k systematic samples (in our survey k was 3). Intervals are determined by $d = \sqrt{L \cdot W/(a \cdot n'_i)}$, where L and W are the length and width of the study site (m), and a is the quadrat area (m²). (Intervals will often need to be rounded.) The units for the interval d are quadrats; however, to calculate the interval directly in meters, use $d' = \sqrt{L \cdot W/n'_i}$. We anticipated the affected area to be 20,000 m² (100 m by 200 m), set sample size at 600 quadrats, and chose 3 random starts. Thus, there were 200 quadrats in each of the 3 systematic samples, and we separated quadrats by 10 m (or 20 quadrats) in the across-river and upstream–downstream directions.

Sample size is the total number of quadrats, both surface and excavated. In general, sample size depends on mussel density; the lower the density, the higher the sample size needed to achieve the desired precision. Because of the negligible effect of the finite population correction in mussel surveys, the study site area is not an important determinant of sample size, at least if the study site area is ≥500 m² and the sampling fraction is ≤0.35 (Smith et al. 2001). (Sampling fraction is the ratio of sample size to population size, i.e., number of quadrats sampled to total number of quadrats possible in the study site.) We considered 3 criteria to determine sample size: coefficient of variation (CV), margin of error/1000 m² (MOE), and probability of encountering a species given it is present at the site ($1-\beta$). Margin of error is 2 SE for estimates of abundance/1000 m². (Margin of error is used commonly in reporting results of opinion polls, and we used it as a planning device, but do not recommend its use as a confidence interval [CI] width.) Formulae to calculate CV, MOE, and CI are presented in the Appendix. To calculate β, we used results of Green and Young (1993), which were adapted to the double sampling design (Appendix). Sample size calculations require prior knowledge of variances, which may be available from pilot surveys or surveys at similar sites. However, this limitation caused Smith et al. (2001) to fit a regression relationship between CV and density using available data, and we made use of this approximate relationship to guide sample size at West Hickory.

We determined sample size for *P. clava* because we anticipated it was the species of interest with the lower density and, therefore, the more difficult for which to estimate abundance. We found *P. clava* at a density ~0.10/m² in the Allegheny River at Kennerdell. Thus, based on the relationship between CV and density (Smith et al. 2001), we predicted a sample size of 600 would result in CV ≅ 0.37, MOE ≅ 75, and $1-\beta$ ≅ 0.96.

We recorded surface and buried mussels separately. We removed the surface animals, excavated quadrats to 10 cm or to hardpan, and sifted substrate through a 6.35-mm-mesh screen. Smaller mussels were not captured in our sampling, and are thus not included in our popu-

TABLE 1. Total density and abundance of mussels within the area of direct impact at the West Hickory bridge site on the Allegheny River, July 1999.

Species	Relative abundance (%)	Density (no./m²)	SE	95% CI	Abundance (no./18,600 m²)	SE	95% CI
All		2.810	0.2261	2.400–3.290	52,266	4204.63	44,642–61,192
Actinonaias ligamentina	28.72	0.807	0.1101	0.618–1.055	15,019	2048.06	11,497–19,621
Alasmidonta marginata	0.25	0.007	0.0124	0.0002–0.218	132	231.12	4–4054
Elliptio dilatata	29.25	0.822	0.1306	0.603–1.123	15,300	2428.87	11,209–20,885
Epioblasma torulosa rangiana	17.54	0.493	0.0942	0.339–0.717	9173	1751.43	6309–13,336
Fusconaia subrotunda	0.25	0.007	0.0124	0.0002–0.218	132	231.12	4–4054
Lampsilis cardium	0.78	0.022	0.0218	0.003–0.155	407	406.06	57–2879
L. fasciola	0.75	0.021	0.0123	0.007–0.066	397	228.43	128–1226
L. siliquoidea	0.78	0.022	0.0218	0.003–0.155	407	406.06	57–2879
Lasmigona costata	0.75	0.021	0.0124	0.007–0.067	397	231.12	127–1243
Ligumia recta	1.57	0.044	0.0308	0.011–0.174	813	572.67	204–3233
Pleurobema clava	13.42	0.377	0.0869	0.240–0.592	7010	1615.66	4462–11,013
P. sintoxia	2.06	0.058	0.0332	0.019–0.178	1079	617.58	352–3313
Ptychobranchus fasciolaris	1.53	0.043	0.0214	0.016–0.114	794	398.10	297–2121
Strophitus undulatus	2.35	0.066	0.0376	0.021–0.202	1220	699.44	396–3753

lation estimates. All mussels were replaced in the substrate.

Data analysis

We estimated density and abundance using a regression estimator (Appendix). We used kriging, a statistical technique for spatial prediction, to map the distribution of mussels at the surface based on the sample of quadrats (Thompson 1992). We used GS$^{+}_{TM}$ version 3.1 (Gamma Design Software, Plainwell, Michigan) to generate the spatial predictions and ArcView® GIS version 3.1 (Environmental Systems Research Institute Inc., Redlands, California) to map the predictions generated through kriging.

Results

Qualitative sampling involved 12 biologists searching ~100 person h over 1.5 d. Sixteen biologists (12 observers and 4 data recorders) participated in the quantitative sampling over 2 d. Approximately 80% of sampling was conducted while snorkeling and 20% while SCUBA diving.

Qualitative sampling in areas of direct and indirect effects

Recent shell material was found in 11 middens, which included 1392 shells from 15 species. We found 17 species during the timed search of the direct- and indirect-effects areas; live mussels occurred in all 24 cells. The dominant species were *Actinonaias ligamentina* and *Elliptio dilatata. Epioblasma t. rangiana* and *P. clava* were encountered frequently. The remaining species were present in lower numbers. *Epioblasma t. rangiana* was present in all 24 cells. *Pleurobema clava* was present in 22 cells and was not detected in cells that were 50 to 100 m from the right bank and within 50 m of the bridge. In general, few individuals and species were found 50 to 100 m from the right bank in the deep, fast current (Fig. 1). We found *Fusconaia subrotunda* only in the river channel upstream of the bridge.

Quantitative sampling of the area of direct effects

We sampled 562 quadrats and excavated 183 of those in the direct-effects area. Estimates of total density, including surface and buried mussels are shown in Table 1. The CVs were 19% for *E. t. rangiana* and 23% for *P. clava.*

We found a wide range of sizes, including some small individuals, which indicated that the 2 federally endangered species reproduced recently at the study site (Fig. 2). *Epioblasma t. rangiana* ranged from 12.5 mm to 67.9 mm, and 25% were ≤28.3 mm. The smallest sexually di-

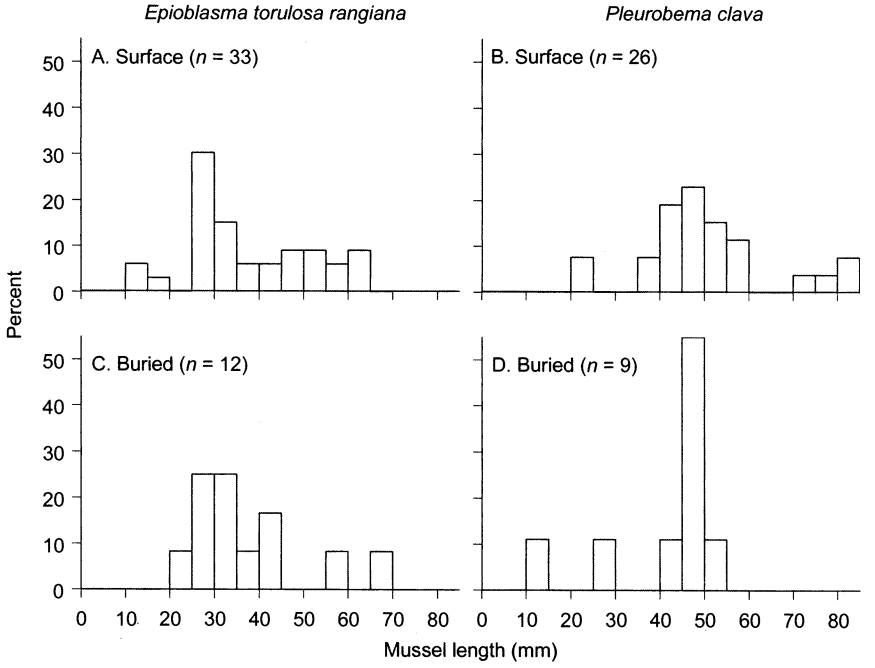

FIG. 2. Size distributions for surface (A) and buried (C) *Epioblasma torulosa rangiana* and surface (B) and buried (D) *Pleurobema clava* in the Allegheny River at West Hickory, Pennsylvania, July 1999. Size distributions are shown separately for those observed at the substrate surface and by excavation of substrate to 10 cm. Numbers of mussels are shown in parentheses; those at the substrate surface include mussels found in quadrats that were not excavated.

morphic *E. t. rangiana* was 28.2 mm, so we classified *E. t. rangiana* <28 mm as juvenile or indeterminate sex. *Pleurobema clava,* which is not sexually dimorphic, ranged from 13.2 mm to 81.9 mm, and 25% were ≤42.6 mm.

Spatial distributions of the 2 federally endangered species at the substrate surface within the area of direct effects are predicted in Fig. 3. The distributions show spatial clustering and incomplete overlap between species. We thought the excavated quadrats alone did not comprise a sufficient sample for spatial prediction.

Detectability of mussels

Detection of mussels at the surface varied across species and was low for some species. For example, only 31% of *P. clava* and 52% of *E. t. rangiana* were detected at the surface. In contrast, >70% of *A. ligamentina* was observed at the surface. In addition to species differences, detection at the surface was sex-specific for *E. t. rangiana*: 80% of females, but only 45% of males were detected at the surface.

Species detectability differed among observation methods and caused bias in relative abundances (Table 2). Ranked abundance varied less than relative abundance. The 2 federally listed species were 3rd, 4th, or 5th most abundant and *A. ligamentina* and *E. dilatata* were usually 1st or 2nd. *Epioblasma t. rangiana* was overrepresented in the middens (ranked 2nd). The ranked abundances of several other species changed dramatically among observation methods. Because it was relatively difficult to detect, *Strophitus undulatus,* which tied for 10th based on surface counts and had a similar ranking in the midden search, was actually the 5th most abundant species. Because of its high detectability, *Ligumia recta* was 3rd based on the timed search, but was actually the 7th most abundant species.

Estimated sex ratio for *E. t. rangiana* depended on observation method because adult females were more likely to be on the surface and more visible. Based on timed searches, 60% of the *E. t. rangiana* population was adult female, the rest being adult male or juvenile. However, based on surface counts using quadrats, 30% were adult

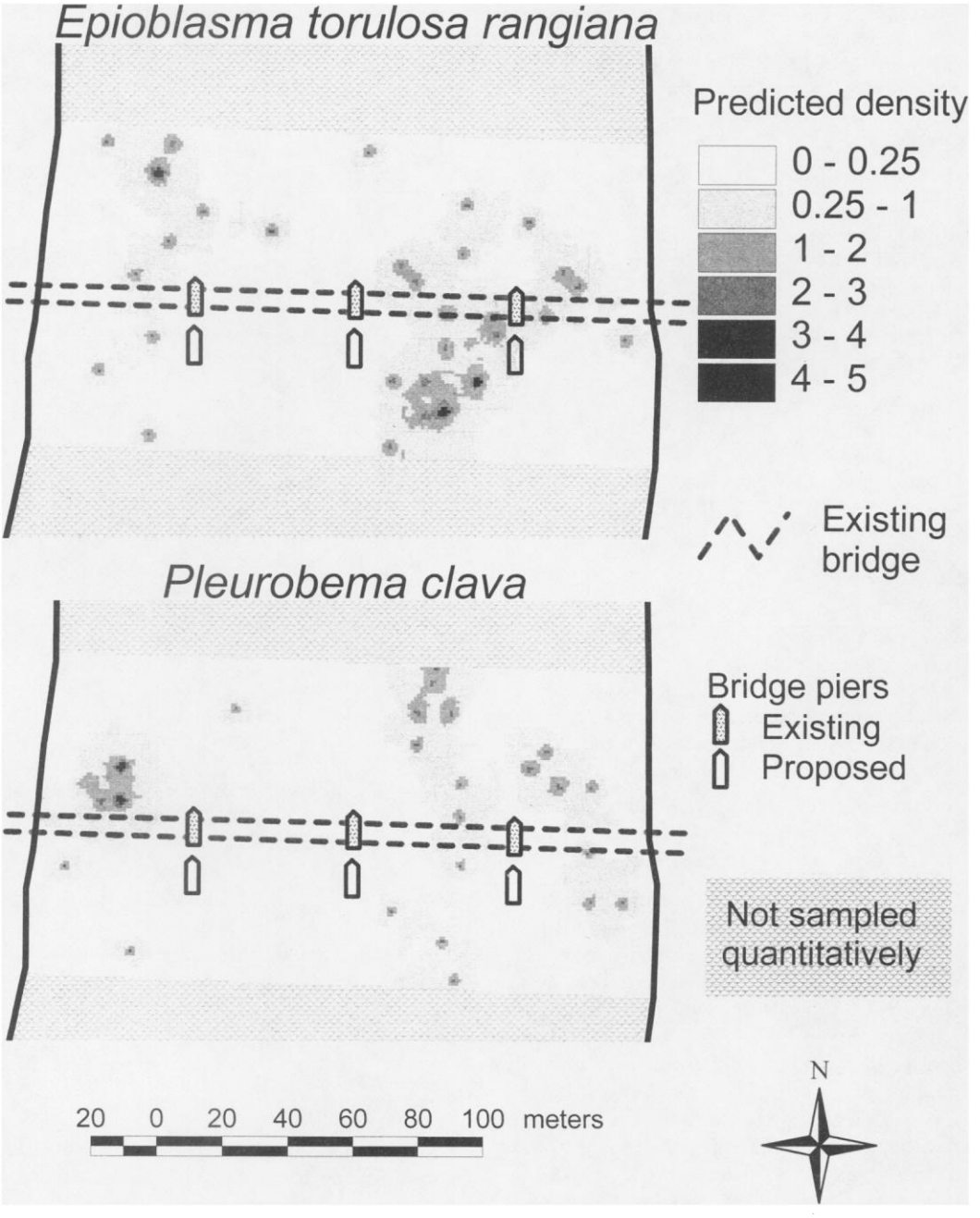

FIG. 3. Predicted spatial distribution of *Epioblasma torulosa rangiana* and *Pleurobema clava* at the substrate surface in the Allegheny River at West Hickory, Pennsylvania, July 1999. Density (no./m²) was predicted using kriging from a systematic sample of 562 0.25-m² quadrats over an area of 18,600 m².

TABLE 2. Relative and ranked abundance based on excavated quadrats, surface counts in quadrats, timed searches, and a midden search that were conducted within 50 m of the existing bridge at West Hickory on the Allegheny River, July 1999. – = species not detected.

Species	Relative abundance (%)				Ranked abundance			
	Excavated quadrats	Surface counts	Timed searches	Midden search	Excavated quadrats	Surface counts	Timed searches	Midden search
Actinonaias ligamentina	28.72	35.16	54.56	11.21	2	1	1	4
Alasmidonta marginata	0.25	0.46	0.04	0.07	13.5	12.5	16	15
Amblema plicata	–	–	0.01	–	–	–	17	–
Elliptio dilatata	29.25	26.03	21.62	35.56	1	2	2	1
Epioblasma torulosa rangiana	17.54	15.07	5.21	23.92	3	3	4	2
Fusconaia subrotunda	0.25	0.46	0.12	0.22	13.5	12.5	14	13
Lampsilis cardium	0.78	0.91	0.61	0.29	9.5	10	11	11.5
L. fasciola	0.75	1.37	0.57	0.93	11.5	7.5	12	7
L. ovata	–	–	0.08	0.36	–	–	15	9.5
L. siliquoidea	0.78	–	1.83	0.14	9.5	–	7	14
Lasmigona costata	0.75	1.37	0.65	0.29	11.5	7.5	9.5	11.5
Ligumia recta	1.57	2.74	6.39	0.79	7	5.5	3	8
Pleurobema clava	13.42	11.87	4.32	18.46	4	4	5	3
P. sintoxia	2.06	0.91	0.65	2.23	6	10	9.5	5
Ptychobranchus fasciolaris	1.53	2.74	2.24	1.36	8	5.5	6	6
Strophitus undulatus	2.35	0.91	0.94	0.36	5	10	8	9.5
Villosa fabilis	–	–	0.16	–	–	–	13	–

females, and based on surface and buried mussels in excavated quadrats, 20% of the *E. t. rangiana* population was adult female.

Sample size for future surveys

We calculated sample sizes using variance observed for *E. t. rangiana* and *P. clava* at 9 sites on the Allegheny River and French Creek where we implemented the double sampling design. Density at these sites, which included West Hickory, ranged from 0.08 to 1.5/m^2; 75% of the species–site combinations were <1.1/m^2 with a median of 0.64/m^2. The relationship between CV and density given sample size was strong, negative, and linear for density on a transformed scale of $1/\sqrt{\text{density}}$ (R^2 = 0.86). We used this relationship to calculate CV and MOE (Table 3); these values can be used for planning future surveys of *E. t. rangiana* and *P. clava* in the Allegheny River drainage. For example, if a species of interest was expected to occur at 0.10/m^2, then a sample size of 500 would assure a CV = 0.41, MOE = 83/1000 m^2, and a 93% chance that at least 1 individual would be detected.

Discussion

It is US public policy that biological assessments "should always use the best available scientific and commercial data to make findings regarding ... effects of a proposed action on the species or critical habitat" (US Fish and Wildlife Service and National Marine Fisheries Service 1998:xxi). This statement also applies to freshwater mussels, so statistically valid site-specific surveys are needed to correctly determine potential impacts on federally listed species.

Survey protocol for freshwater mussels

We combined qualitative and quantitative sampling approaches in our protocol because neither method alone was sufficient to meet all objectives. The timed search is generally efficient (less costly) at detecting the presence of rare species (Miller and Payne 1993, Strayer et al. 1997, Vaughn et al. 1997). Timed searches, however, are inappropriate for determining relative abundance of species and may actually provide misleading information by overestimating the abundance of some species and under-

TABLE 3. Calculations of sample size based on a double sampling design and data from surveys of *Epioblasma torulosa rangiana* and *Pleurobema clava* at 9 sites in the Allegheny River. Coefficient of variation (CV), margin of error/1000 m² (MOE), and probability of encountering an individual (1 − β) vary by density (no./m²) and number of quadrats sampled. MOE is 2*SE for the estimate of no./1000 m².

Density	Sample size (no. of quadrats)																	
	200			300			400			500			600			700		
	CV	MOE	1 − β	CV	MOE	1 − β	CV	MOE	1 − β	CV	MOE	1 − β	CV	MOE	1 − β	CV	MOE	1 − β
0.05	0.92	92	0.46	0.75	75	0.58	0.65	65	0.68	0.58	58	0.75	0.53	53	0.80	0.49	49	0.85
0.10	0.65	131	0.68	0.53	106	0.80	0.46	92	0.88	0.41	83	0.93	0.37	75	0.96	0.35	70	0.97
0.15	0.53	160	0.80	0.43	129	0.91	0.38	113	0.96	0.34	101	0.98	0.31	93	0.99	0.29	86	0.99
0.20	0.46	186	0.88	0.37	149	0.96	0.33	131	0.98	0.29	117	0.99	0.27	107	0.99	0.25	99	0.99
0.25	0.42	207	0.93	0.33	167	0.98	0.29	147	0.99	0.26	132	0.99	0.24	120	0.99	0.22	111	0.99
0.30	0.38	228	0.96	0.31	183	0.99	0.27	161	0.99	0.24	144	0.99	0.22	132	0.99	0.20	122	0.99
0.40	0.33	264	0.98	0.26	211	0.99	0.23	186	0.99	0.21	167	0.99	0.19	152	0.99	0.18	141	0.99
0.50	0.30	296	0.99	0.24	236	0.99	0.21	209	0.99	0.19	187	0.99	0.17	171	0.99	0.16	158	0.99
0.60	0.27	325	0.99	0.22	259	0.99	0.19	230	0.99	0.17	206	0.99	0.16	188	0.99	0.14	174	0.99

estimating the abundance of others (Miller and Payne 1993, Vaughn et al. 1997, Smith et al. 2001). The combination of surface counts and excavation in the double sampling design allows increased spatial coverage while estimating mussel densities free from the biases of detectability, which affect qualitative methods. The regression estimator used with the double sampling design is based on an approximate linear relationship between surface and total counts, and this assumption should be verified during analysis. The double sampling design balances the cost and benefits of excavation, but does add complexity to data analysis (Appendix). We believe the complexity of analysis is more than compensated for by greater spatial coverage for fixed cost compared to a survey with 100% excavation. However, if all quadrats are excavated, then the design becomes a straightforward systematic sampling design, the simplicity of which may be preferable.

Our decision to use 0.25-m^2 quadrats as the sampling unit was guided by sampling efficiency. We considered the best sampling unit as one that results in the most reliable (least variable) estimate of population density or abundance. It is clear from the literature that for clustered populations, such as freshwater mussels (Downing and Downing 1992), the smaller the sampling unit the more reliable the estimate of population size or density (see Elliott 1977 and citations therein). However, there is a limit to this recommendation; a unit can be so small that errors in deciding whether an organism is inside the unit can exceed reductions in sampling variance. In our opinion, 0.25-m^2 quadrats are small enough to benefit from the reduced variance, but not so small that boundary errors dominate.

Our protocol can be adapted to meet specific conditions. Logistics, especially, will be determined on a case-by-case basis. For example, boundaries of the site will depend on construction methods, and how boundaries are marked will depend on the configuration of the site. Some may find our quantitative sampling protocol too complicated or costly; however, we stress that reliable and credible estimates of abundance of rare mussels require a substantial effort. Given that the survey at West Hickory took 3.5 d to complete, we believe the protocol is practical and provides a useful framework and starting point.

Stratification would improve the protocol in certain cases. For example, cells could be stratified into high- and low-density strata, depending on results from the timed search, and sampling effort could be allocated directly proportional to variance. Alternatively, cells could be stratified into wadeable and deep-water strata, and sampling effort could be allocated inversely proportional to cost to account for the higher cost of SCUBA diving. Formulae to estimate density or abundance must be adjusted accordingly when stratification or other complexity is incorporated into the protocol.

The regression relationship between density and CV reported by Smith et al. (2001) is based on the double sampling design and surveys at 14 sites of multispecies assemblages, with little data from sites with densities $<1/m^2$. Nevertheless, the predictions of CV from this relationship were within a couple percentage points of the predictions of CV using data exclusively from *E. t. rangiana* and *P. clava* where most densities were $<1/m^2$. The closeness of these 2 independent sets of predictions gives us some confidence in recommending the use of the sample size calculations in Table 3 to determine sample size for species other than *E. t. rangiana* and *P. clava.*

Protocol application and interpretation of results

Application of our protocol in the Allegheny River at the West Hickory bridge site revealed an extensive freshwater mussel bed, which included the presence of 2 federally listed species. The abundance of *P. clava* relative to its known distribution and abundance (US Fish and Wildlife Service 1994) made West Hickory a significant site for this species. The predicted spatial distribution of mussels within the area of direct effects showed spatially clustered populations, which is typical for freshwater mussel populations (Kovalak et al. 1986, Downing and Downing 1992). If mussels need to be relocated from the direct-effects area, managers can use the predicted spatial distribution to plan the extent of and allocate effort for relocation.

Reliable and credible site-specific population estimates are just the beginning of an assessment. There remains the problem of interpreting site-specific effects in the context of river-wide (or range-wide) population viability. Suppose that all of the mussels in the direct-effects area die as a result of bridge construction. In that

case, we predict that 9173 (95% CI: 6309–13,336) *E. t. rangiana* and 7010 (95% CI: 4462–11,013) *P. clava* will be lost. How will that amount of mortality affect the viability of populations in the Allegheny River and of the species throughout their ranges? The Allegheny River is presumed to support a sparse and discontinuous distribution of *P. clava* and a more uniform distribution of *E. t. rangiana*; these 2 species persist in few other river systems (US Fish and Wildlife Service 1994). Most of the Allegheny River has not been disturbed by bridge construction and should support comparable mussel populations to those at the few bridge sites where quantitative surveys have been conducted. However, the necessary quantitative surveys have not been done throughout the Allegheny River to support this conclusion/assumption. In the face of this uncertainty, the precautionary principle (Buhl-Mortensen and Welin 1998) requires that potentially damaging impacts be avoided to significant populations of *P. clava* and *E. t. rangiana* in the Allegheny River. Ultimately, the issue returns to availability of best scientific and commercial data, or rather the lack of such information.

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Appendix. Formulae for estimating density and calculating sample size.

Systematic sampling formulae

These formulae apply when estimating surface density or total density if all quadrats are excavated. The underlying sampling design is systematic sampling with multiple random starts. Let M denote the number of possible systematic samples in the site to be sampled, and let m denote the number of random starts:

$$M = \frac{A}{a}\frac{m}{\sum_{i=1}^{m} n_i}$$

where A is the area of the site, a is the area of the sampling unit (e.g., 0.25-m² quadrat), and n_i is the number of quadrats in the i^{th} systematic sample. Let x_i be the sum of the counts for all quadrats in the i^{th} systematic sample. An unbiased estimator of population abundance is:

$$\hat{T} = \frac{M}{m}\sum_{i=1}^{m} x_i.$$

An estimate of variance for $\hat{T}$ is:

$$\widehat{\text{var}}(\hat{T}) = \frac{M(M-m)}{m}\frac{\sum_{i=1}^{m}(x_i - \bar{x})^2}{m-1}$$

where $\bar{x} = (1/m)\,\Sigma_{i=1}^{m} x_i$. An estimate of density (no./m²) is calculated by $\hat{\mu} = \hat{T}/A$. An estimate of variance for $\hat{\mu}$ is $\widehat{\text{var}}(\hat{\mu}) = (1/A)^2\,\widehat{\text{var}}(\hat{T})$.

It is sometimes possible that the survey results in $x_i > 0$ and equal for all m systematic samples. If so, the estimate of variance will equal 0, and we suggest estimating variance assuming that the $n = \Sigma_{i=1}^{m} n_i$ quadrats were selected by simple random sampling rather than by systematic sampling with multiple starts. This solution is conservative in the sense that variance for $\hat{T}$ will tend to be overestimated (P.S. Pooler, US Geological Survey, Kearneysville, West Virginia, and D.R. Smith, unpublished data). The formulae for $\hat{T}$, $\hat{\mu}$, and $\widehat{\text{var}}(\hat{\mu})$ are unchanged. However, the estimate of variance for $\hat{T}$ becomes:

$$\widehat{\text{var}}(\hat{T}_{SRS}) = \frac{N(N-n)}{n}\frac{\sum_{i=1}^{m}\sum_{j=1}^{n_i}(x_{ij} - \bar{x})^2}{n-1}$$

where N is the number of possible quadrats in

the site (i.e., A/a), and x_{ij} is the surface count for the j^{th} quadrat in the i^{th} systematic sample.

Regression estimator for incorporating excavated quadrats in estimates of abundance

These formulae apply when estimating total density if a representative portion of the quadrats in the sample is excavated (Smith et al. 2001). The underlying sampling design is double sampling. A simple linear regression model is fit to the data from the excavated quadrats. In this regression, the total count is the response variable (y) and the surface count is the explanatory variable (x). Provided that the relationship between surface and total counts is approximately linear, the regression model can be used to calibrate surface counts and estimate density. This assumption was met for a variety of species at West Hickory and elsewhere (Smith et al. 2001); however, the assumption should be verified routinely during analysis. Formulae for estimating population density ($\hat{\mu}_{lr}$) using the regression estimator under the double sampling design are (Hedayat and Sinha 1991, Thompson 1992):

$$\hat{\mu}_{lr} = a^{-1}[\bar{y}_2 - \hat{\beta}_1(\bar{x}_2 - \bar{x}_1)]$$

with variance

$$\widehat{\text{var}}(\hat{\mu}_{lr}) = a^{-2}\left\{\left(\frac{N-n'}{N}\right)\frac{s^2}{n'} + \left[\frac{n'-n}{n'n(n-2)}\right]\sum_{i=1}^{n}(y_i - \hat{\beta}_0 - \hat{\beta}_1 x_i)^2\right\}$$

where a is the quadrat area, $\bar{y}_2$ is the mean total count from the excavated subsample, $\bar{x}_1$ and $\bar{x}_2$ are the mean surface counts from the 1st sample and excavated subsample, $\hat{\beta}_0$ and $\hat{\beta}_1$ are estimates of the regression parameters, s^2 is the variance of total counts in the excavated subsample, N is the total number of quadrats at a site (i.e., A/a), n' is the sample size of the 1st sample, and n is the number of excavated quadrats. The variance s^2 can be estimated from the systematic sample of excavated quadrats by (Thompson 1992):

$$\hat{s}^2 = \frac{M(\bar{n}_i - 1)s_w^2 + (M-1)\bar{n}_i s_b^2}{M\bar{n}_i - 1}$$

where M is the number of systematic samples in the population, n_i is the number of quadrats in the i^{th} systematic sample ($\bar{n}_i$ is the mean of the n_i), $s_w^2 = \sum_{i=1}^{m}\sum_{j=1}^{n_i}(x_{ij} - \bar{x}_i)^2/[m(\bar{n}_i - 1)]$, $s_b^2 = \sum_{i=1}^{m}(x_i - \bar{x})^2/(m-1)$ and $\bar{x}_i = \sum_{j=1}^{n_i} x_{ij}/n_i$. An estimate of population abundance is simply $\hat{T}_{lr} = A \times \hat{\mu}_{lr}$ with an estimate of its variance $\widehat{\text{var}}(\hat{T}_{lr}) = A^2\widehat{\text{var}}(\hat{\mu}_{lr})$.

Calculation of confidence intervals (CI)

Based on simulations of sampling mussel populations (P. S. Pooler and D. R. Smith, unpublished data), the sampling distributions for the estimators of population total and density are not normally distributed and tend to be skewed right. A simple logarithmic transformation of the estimates usually results in CIs with coverage close to nominal. For example, we calculated approximate 95% CIs for population abundance by:

$$\exp\left(\log(\hat{T}) \pm 1.96 \cdot \sqrt{\frac{\text{var}(\hat{T})}{\hat{T}^2}}\right).$$

Sample size calculations

By removing the finite population correction, the variance of density estimate (i.e., var[$\hat{\mu}_{lr}$]) under the double sampling design can be written:

$$\text{var}(\hat{\mu}_{lr}) \le \left[\frac{(s^2 - s_{lr}^2)f_2 + s_{lr}^2}{n'a^2 f_2}\right]$$

where n' is the sample size or number of quadrats, s^2 is the variance of total counts among quadrats, f_2 is the fraction of the sample size that is excavated ($f_2 = n/n'$), s_{lr}^2 is the mean square error from the regression ($s_{lr}^2 = \sum_{i=1}^{n}(y_i - \hat{\beta}_0 - \hat{\beta}_1 x_i)^2/(n-2)$, and a is the quadrat area. We have found that the above inequality is very close to an equality at least for study site areas >500 m^2 and sampling fractions <0.35. Thus, we use this simpler, albeit approximate, variance formula to calculate sample size.

To achieve a desired CV, say CV_0, the sample size formula is:

$$n' = \left(\frac{1}{CV_0\mu}\right)^2\left[\frac{(s^2 - s_{lr}^2)f_2 + s_{lr}^2}{a^2 f_2}\right].$$

If the objective is to achieve a desired margin of error/1000 m^2 (say MOE_0), the sample size formula is:

$$n' = \left(\frac{2000}{MOE_0}\right)^2\left[\frac{(s^2 - s_{lr}^2)f_2 + s_{lr}^2}{a^2 f_2}\right].$$

Based on Green and Young (1993), if the objective is to control the probability of failing to detect a species in n' quadrats, the sample size formula is:

$$n' = \frac{-4 \ln(\beta_0)}{\mu[(1 - f_2)\lambda + f_2]}$$

where β_0 is the acceptable risk of not detecting the species and λ is the proportion of the species at the substrate surface.

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